



## Physiology

# Predawn respiration rates during flowering are highly predictive of yield response in *Gossypium hirsutum* when yield variability is water-induced



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## ABSTRACT

Respiratory carbon evolution by leaves under abiotic stress is implicated as a major limitation to crop productivity; however, respiration rates of fully expanded leaves are positively associated with plant growth rates. Given the substantial sensitivity of plant growth to drought, it was hypothesized that predawn respiration rates ( $R_{PD}$ ) would be (1) more sensitive to drought than photosynthetic processes and (2) highly predictive of water-induced yield variability in *Gossypium hirsutum*. Two studies (at Tifton and Camilla Georgia) addressed these hypotheses. At Tifton, drought was imposed beginning at the onset of flowering (first flower) and continuing for three weeks (peak bloom) followed by a recovery period, and predawn water potential ( $\Psi_{PD}$ ),  $R_{PD}$ , net photosynthesis ( $A_N$ ) and maximum quantum yield of photosystem II ( $F_v/F_m$ ) were measured throughout the study period. At Camilla, plants were exposed to five different irrigation regimes throughout the growing season, and average  $\Psi_{PD}$  and  $R_{PD}$  were determined between first flower and peak bloom for all treatments. For both sites, fiber yield was assessed at crop maturity. The relationships between  $\Psi_{PD}$ ,  $R_{PD}$  and yield were assessed via non-linear regression. It was concluded for field-grown *G. hirsutum* that (1)  $R_{PD}$  is exceptionally sensitive to progressive drought (more so than  $A_N$  or  $F_v/F_m$ ) and (2) average  $R_{PD}$  from first flower to peak bloom is highly predictive of water-induced yield variability.

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## Introduction

Mechanisms of drought-induced photosynthetic declines have been extensively studied, and although often the topic of lively debate (Medrano et al., 2002; Flexas et al., 2004; Lawlor and Tezara, 2009), a few widely accepted conclusions can be drawn from the literature. In contrast, a consensus view of the impact of drought on respiration ( $R$ ) is not as easily obtained. For example, most reports indicate that constraints to  $\text{CO}_2$  diffusion from the atmosphere to the chloroplast are the primary limitation to net photosynthesis ( $A_N$ ) under mild to moderate drought stress

(Chastain et al., 2014; Ennahli and Earl, 2005; Flexas et al., 2006; Medrano et al., 2002; Snider et al., 2014). Only under conditions of extreme water deficit ( $g_s \leq 0.05 \text{ mmol m}^{-2} \text{ s}^{-1}$ ) do non-diffusional limitations to  $A_N$  become apparent either due to photosynthetic downregulation or damage to the photosynthetic apparatus (Flexas et al., 2006; Medrano et al., 2002). Although there is also some evidence that decreased ATP production represents a major limitation to  $A_N$  (Lawlor and Tezara, 2009; Tezara et al., 1999), most available literature on field-grown *Gossypium hirsutum* supports the view that the thylakoid reactions are either unaffected or respond positively to mild or moderate drought stress (Chastain et al., 2014; Kitao and Lei, 2007; Snider et al., 2014; Zhang et al., 2011).

When considering respiration rates ( $R$ ) in response to drought, there is a stark contrast between the consensus view that drought generally decreases the rate of respiration in whole plants and

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roots (Atkin and Macherel, 2009; Earl et al., 2012) and the seemingly disparate observations that respiration rates in individual leaves may decrease, increase, or remain unchanged in response to water deficit (Atkin and Macherel, 2009; Flexas et al., 2006; Galmes et al., 2007). The explanation for a lack of consensus in the literature is that  $R$  exhibits a biphasic response to drought, initially declining in response to the onset of drought, then increasing under the most severe water deficit conditions (Atkin and Macherel, 2009; Flexas et al., 2006). Therefore, the timing, duration, and severity of drought at the time of measurement will influence whether positive or negative responses of  $R$  to water deficit are observed. Because the magnitude of change in  $R$  (positive or negative) is small relative to the large declines in photosynthesis observed under drought,  $R$  nearly always increases as a proportion of photosynthetic carbon fixation (Atkin and Macherel, 2009). This has led many authors to suggest that a disruption in carbon balance, driven by the contrasting sensitivities of photosynthesis and respiration, may be a major contributor to decreased productivity under drought (Atkin and Macherel, 2009; Chastain et al., 2014; Flexas et al., 2006; Lawlor, 1976; Lawlor and Cornic, 2002).

High  $R$  is often implicated as a major yield-limiting process under a range of conditions. For example, it has been suggested that crop yields could be improved if agronomically important plant species were selected for low respiration rates (Earl and Tollenaar, 1998; Earl et al., 2012; Zhu et al., 2010). High  $R$  in response to elevated temperature has been credited with decreased productivity in rice (Mohammed and Tarpley, 2009; Peng et al., 2004) and with drought-induced tree mortality via carbon starvation (Adams et al., 2009; Sevanto et al., 2014). It is important to note, however, respiration is essential for both growth and maintenance (Johnson, 1990), where rapidly growing plant species often exhibit higher  $R$  in mature leaves than slow growing species (Galmes et al., 2007). Furthermore, photosynthesis and cellular respiration are interdependent processes (Atkin and Macherel, 2009; Millar et al., 2011), and nighttime  $R$  has been strongly correlated with daytime  $A_N$  in oak leaves (Whitehead et al., 2004). Finally, drought-induced respiration responses of fully expanded leaves are generally attributed to impacts on the maintenance component of respiration (Atkin and Macherel, 2009). However, nocturnal  $R$  in mature leaves has been positively correlated with nighttime translocation rates in soybean (Bunce, 2007), indicating that high “maintenance”  $R$  may actually be indicative of high translocation rates and sink demand at other locations on the plant.

For *G. hirsutum* (Upland cotton), fiber yield is particularly sensitive to drought stress extending from the onset of flowering (referred to as first flower in cotton) until 2 to 3 weeks later (peak bloom) and requires more water at this growth stage than at any other phenological development period (Bauer et al., 2012), where exposure to water deficit during this period can drastically limit yield (Dumka et al., 2004). However, the potential for  $R$  to predict water-induced yield variability is relatively unexplored. The objectives of the current study were (1) to characterize predawn water potential ( $\Psi_{PD}$ ), predawn respiration ( $R_{PD}$ ),  $A_N$ , and maximum quantum yield of photosystem II ( $F_v/F_m$ ) for field-grown *G. hirsutum* during progressive drought and recovery and (2) to quantify the relationship between  $R_{PD}$  measured from first flower to peak bloom and fiber yields when yield variability was water induced. Because respiration rates are often related to plant growth rates and growth represents the most sensitive physiological process to water deficit, it was hypothesized (1) that  $R_{PD}$  in *G. hirsutum* would be more negatively impacted during the onset of drought at flowering than photosynthetic processes and (2) that average  $R_{PD}$  from first flower to peak bloom would be highly predictive of water-induced yield variability.

## Materials and methods

### Experiment 1: Tifton study

To address the impact of progressive drought stress on physiological and yield responses in cotton, seeds of three cotton cultivars [*Gossypium hirsutum* cv. DP 1050 B2RF; FM 1944 GLB2; PHY 499 WRF] were sown by hand at a 0.91 m inter-row spacing, 10 seeds per meter seeding rate, and at 2.5 cm planting depth on June 5, 2014 in Tifton, GA (31°28'32"N, 83°31'43"W). Field plots were two rows  $\times$  1.8 m and were arranged using a completely randomized design ( $n=4$  for each cultivar). All plots were maintained well-watered according to the University of Georgia Cooperative Extension Service “Checkbook” approach (Collins et al., 2013) until first flower (July 31, 2014). The term “first flower” refers to the phenological stage of crop development at which 50% of the plants in the current study possessed at least one open flower. At this time, irrigation ceased and a large rain-exclusion shelter with a clear plastic covering (6 mil UV stabilized greenhouse poly covering from Atlas Manufacturing, Alapaha, GA; 88–91% light transmission) was placed over the entire study to prevent rainfall from reaching the crop surface for approximately 3 weeks (Drought period ended on August 21). Thus, drought was initiated at first flower and allowed to progress until peak bloom (typically defined as two to three weeks after first flower). Subsequently, the shelter was removed and plants were returned to well-watered conditions. Measurements of predawn water potential ( $\Psi_{PD}$ ), predawn respiration ( $R_{PD}$ ), net photosynthesis ( $A_N$ ), and maximum quantum yield of photosystem II ( $F_v/F_m$ ) were conducted throughout the drought stress period and following a four day recovery period; whole-plot fiber yield was determined at crop maturity.

### Experiment 2: Camilla study

A concurrent field study was established to assess the physiological and yield response of cotton to a wide range of plant water status through variation in irrigation regime. At a field site near Camilla, GA (USA) (31°16'48"N, 84°17'29"W), seeds of *G. hirsutum* cv. PHY 499 WRF (Dow AgroSciences) and FM 1944 GLB2 (Bayer CropScience) were sown at a 2.5 cm depth on May 6, 2013 and June 2, 2014. A 0.91 m inter-row spacing was used and a seeding rate of 11 seeds  $m^{-1}$  row. Plots were six rows wide and 12.2 m long with 2.4 m bare-soil alleys. The soil type at the Camilla study site is a Lucy loamy sand (loamy, kaolinitic, thermic Arenic Kandicudults). Seedbed preparation, fertilization, and pest management was conducted according to University of Georgia Cooperative Extension Service recommendations (Collins et al., 2013). Prior to irrigation treatment initiation at the appearance of the first floral bud (square), uniform stand establishment was obtained for all treatments by supplementing rainfall with sprinkler irrigation (18.5 cm rainfall + irrigation in 2014 prior to squaring).

Five different irrigation treatments were initiated on July 7, 2014. **T1:** plants maintained well-watered using the checkbook method recommended by University of Georgia Cooperative Extension Service. Using this approach, irrigation supplements rainfall to provide a weekly water requirement for a given stage of crop development. **T2:** irrigation triggered when the average predawn leaf water potential ( $\Psi_{PD}$ ; measured three times per week) for this treatment declined below  $-0.5$  MPa. **T3:** irrigation triggered when the average  $\Psi_{PD}$  for this treatment declined below  $-0.7$  MPa. **T4:** irrigation triggered when the average  $\Psi_{PD}$  for this treatment declined below  $-0.9$  MPa. **T5:** No supplemental irrigation provided beyond stand establishment. Plants were irrigated using 30 cm deep subsurface drip lines positioned in alternating row middles. Because  $\Psi_{PD}$  measurements and irrigation decisions were made three times per week (Monday, Wednesday and Friday), irrigation

water was applied to plant-based treatments at 1/3 the full weekly recommended Checkbook rate each time a  $\Psi_{PD}$  threshold was reached for a given plant-based treatment. At first open boll (when open bolls were first observed in the latest maturing irrigation treatment), irrigation was terminated. The experimental design was a randomized complete block split plot design with two cultivars and five irrigation treatments ( $n = 4$ ). Irrigation treatment represented the whole-plot factor and cultivar the split plot factor. Survey measurements of  $R_{PD}$  were conducted for all plots at two key times during the growing season, once on July 26 (during the first week of flowering) and once on August 8 (during the peak bloom period). Fiber yield was determined at crop maturity.

#### Predawn water potential measurements

At both the Tifton and Camilla study sites, an uppermost, fully-expanded mainstem leaf (the fourth unfurled leaf node below the apical meristem) was excised from one plant in each plot between 0400 and 0600 h and used for predawn water potential measurements ( $\Psi_{PD}$ ). The petiole was sealed in a compression gasket, and the leaf blade was sealed in a Scholander pressure chamber (PMS Instrument Company, Albany, OR). The chamber was pressurized at a rate of  $0.1 \text{ MPa s}^{-1}$  and the pressure required to bring the water column to the cut surface of the stem was recorded;  $\Psi_{PD}$  values were reported in MPa. Less than 10 s elapsed from when the leaf was severed from the plant to the initial pressurization of the chamber. At the Tifton location  $\Psi_{PD}$  was measured throughout the drought stress period (July 31 and August 4, 7, 12, 19, 21) and following a four day recovery period (August 25). At the Camilla location,  $\Psi_{PD}$  was measured on Monday, Wednesday, and Friday of each week for all treatments since  $\Psi_{PD}$  measurements were used to make irrigation scheduling decisions. To compare the water status of each site, cultivar, and irrigation treatment from first flower to peak bloom, the average  $\Psi_{PD}$  for each plot was determined from July 31 to August 21 for the Tifton location and from July 26 to August 8 for the Camilla location (six measurement times per plot).

#### Chlorophyll *a* fluorescence and net photosynthesis

Chlorophyll *a* fluorescence and net photosynthesis ( $A_N$ ) are reported for the progressive drought study at the Tifton location only. Chlorophyll *a* fluorescence was measured between 0400 and 0600 h (photosynthetically active radiation (PAR) = 0) using a portable pulse amplitude modulation fluorometer (Model OS5p, Opti-Sciences, Hudson, NH). Specifically, maximum quantum yield of photosystem II ( $F_v/F_m$ ) was determined on uppermost, fully expanded, mainstem leaves by first measuring minimum fluorescence intensity ( $F_o$ ) *in situ* under a low-intensity, red modulation light (approximately  $1 \mu\text{mol m}^{-2} \text{ s}^{-1}$  PAR) followed by maximal fluorescence intensity ( $F_m$ ) measurements using a saturating flash of  $15,000 \mu\text{mol m}^{-2} \text{ s}^{-1}$  for 0.8 s.  $F_v/F_m$  was calculated according to Maxwell and Johnson (2000). Afternoon net photosynthetic rates ( $A_N$ ) were measured between 1200 and 1400 h on leaves at the same developmental stage as those sampled for fluorescence analysis using an LI-6400 portable photosynthesis system (Li-Cor, Lincoln, NE). All leaves were measured under chamber irradiance =  $2000 \mu\text{mol m}^{-2} \text{ s}^{-1}$  PAR, reference  $\text{CO}_2$  concentration =  $400 \mu\text{mol mol}^{-1}$ , and a flow rate of  $500 \mu\text{mol s}^{-1}$ . Block temperature was set equal to ambient temperature conditions (as measured by type K thermocouple in a shaded location immediately adjacent to the leaves being measured).  $A_N$  and  $F_v/F_m$  were measured throughout the drought stress period (July 31 and August 4, 7, 12, 19, 21) and following a four day recovery period (August 25).

#### Predawn respiration measurements

At both study sites, pre-dawn, single-leaf respiration ( $R_{PD}$ ) was determined using an LI-6400 portable photosynthesis system (Li-Cor, Lincoln, NE), where all leaves were measured between 0400 and 0600 h at a reference  $\text{CO}_2$  concentration of  $400 \mu\text{mol mol}^{-1}$  and a flow rate of  $500 \mu\text{mol s}^{-1}$ . Air temperature in the plant canopy during predawn hours was measured using a type K thermocouple positioned immediately adjacent to the leaves being measured, and block temperature was set to equal ambient temperature conditions. Steady-state respiration rates were recorded approximately 120 s after the leaf was enclosed in the leaf chamber. At the Tifton site,  $R_{PD}$  was measured 5 times during the progressive drought period between first flower and peak bloom (between July 31 and August 21) and once after a four day recovery period (August 25). At the Camilla site, due to logistical constraints,  $R_{PD}$  was measured once at first flower and once during peak bloom (July 26 and August 8, respectively). To characterize  $R_{PD}$  for each site, cultivar, and irrigation treatment from first flower to peak bloom, the average  $R_{PD}$  for each plot was determined from July 31 to August 21 for the Tifton location and from July 26 to August 8 for the Camilla location.

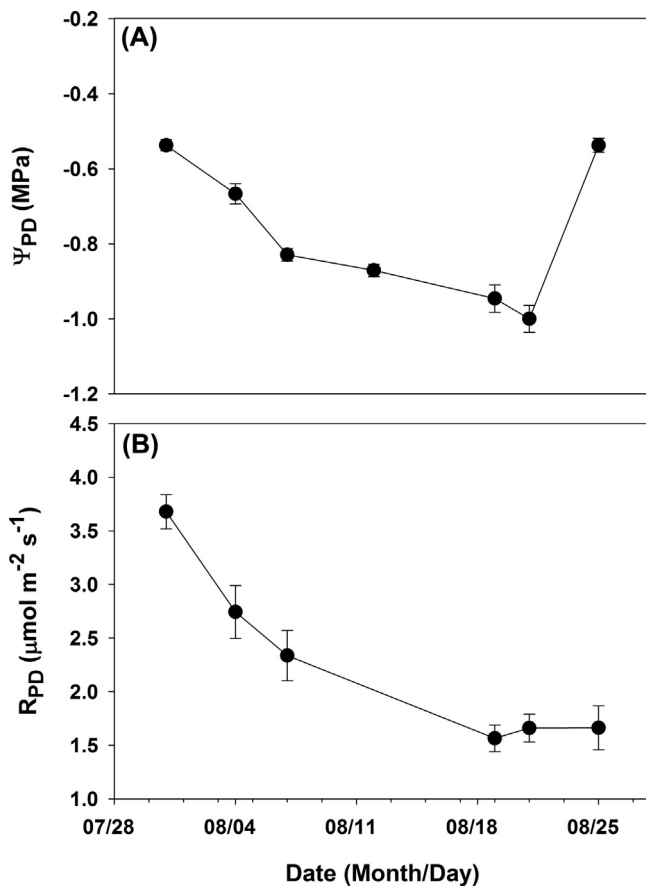
#### Fiber yield determination

To determine end-of-season fiber yields at both locations, harvest aids were applied at 70% open boll to ensure defoliation and complete boll opening. Subsequently the center two rows of each plot were manually (Tifton) or mechanically (Camilla) harvested. A total of 3.6 row meters were harvested at the Tifton location and 24 row meters were harvested at the Camilla location. Hand-harvested seedcotton from the Tifton location was ginned on a 10-saw, laboratory table-top gin, whereas samples from the Camilla location were ginned at the University of Georgia Micro Gin (Tifton, GA). Fiber was then weighed, and fiber yields were extrapolated to  $\text{kg ha}^{-1}$ , based on a 0.91 m inter-row spacing.

#### Statistical analysis

At the Tifton location, during progressive drought, the effect of sample date and cultivar on  $\Psi_{PD}$ ,  $R_{PD}$ ,  $F_v/F_m$ , and  $A_N$  was assessed using a repeated measures analysis of variance, where sample date was the within-subjects factor and cultivar the between-subjects factor. To assess the relationship between the aforementioned physiological parameters and  $\Psi_{PD}$ , the mean ( $n = 12$ )  $R_{PD}$ ,  $F_v/F_m$ , and  $A_N$  for each day during the progressive drought period only (from July 21 to August 21) were plotted versus average  $\Psi_{PD}$ . The recovery data point was excluded from the regression analysis for the Tifton site because it was the aim of the Tifton study to characterize physiological responses to plant water status during the onset of drought. Thus, including the data point would not have addressed this objective. Linear regression was used to quantify the relationship between  $R_{PD}$  and  $\Psi_{PD}$ , whereas second-order polynomial regression was used to assess the relationship between  $A_N$  and  $\Psi_{PD}$  because the whole model fit the data well ( $P < 0.001$ ;  $r^2 = 0.86$ ), and the quadratic component of the regression model was significant ( $P < 0.05$ ).

At the Camilla site, average  $R_{PD}$  and  $\Psi_{PD}$  was first determined for each plot from July 26 to August 8. Subsequently, the effect of irrigation treatment and cultivar on the aforementioned parameters and end-of-season fiber yield was assessed using two-way mixed effects ANOVA according to a split plot randomized complete block design. Blocks represented random effects, whereas irrigation treatment and cultivar were fixed effects. Where significant main effects were observed, mean separation was performed using LSD post hoc analysis. To quantify the dependence of fiber yield in G.



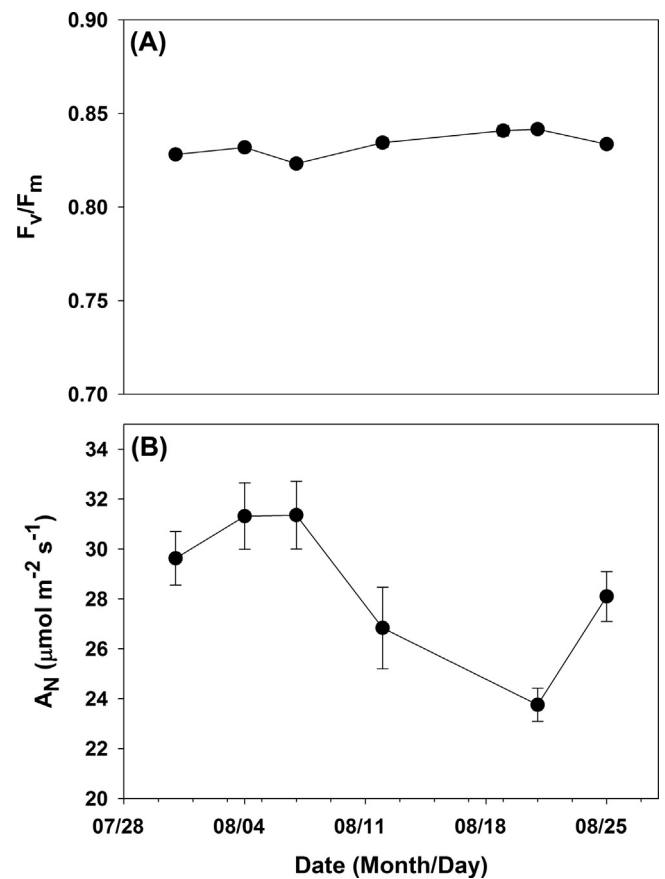
**Fig. 1.** The effect of progressive drought stress and recovery on predawn leaf water potential ( $\Psi_{PD}$ ; A) and respiration ( $R_{PD}$ ; B) at Tifton, Georgia. Water was withheld beginning at first flower (July 31) and continuing until peak bloom (August 21). Thereafter, plants were returned to well-watered conditions for four days prior to a final recovery measurement (August 25). Data represent means and standard errors ( $n = 12$ ).

*hirsutum* upon  $\Psi_{PD}$  and  $R_{PD}$ , variability was first minimized by obtaining the average of each of the aforementioned parameters for a given cultivar  $\times$  irrigation treatment  $\times$  location ( $n = 4$ ). Fiber yield was then plotted versus average  $\Psi_{PD}$  and  $R_{PD}$  and linear regression analysis was conducted. All statistical analyses were conducted using JMP Pro 10 (SAS Institute Inc., Cary, NC) and graphs were constructed using SigmaPlot 12.5 (Systat Software Inc., San Jose, CA). In all instances,  $\alpha = 0.05$ .

## Results

### Physiological responses to progressive drought

For the Tifton study, plants were maintained well-watered as defined using the University of Georgia Cooperative Extension Service "Checkbook" approach until first flower (July 31, 2014). At this point, irrigation ceased, and rainfall was excluded until August 21; plants were then returned to well-watered conditions for four days prior to the last in-season sample date (August 25). Average predawn water potential ( $\Psi_{PD}$ ) was clearly responsive to drought imposition (Fig. 1). For example,  $\Psi_{PD}$  was  $-0.53$  MPa on July 31 and exhibited a non-linear decline throughout the drought period, reaching a minimum of  $-0.97$  MPa on August 21. After the four day recovery period,  $\Psi_{PD}$  had returned to pre-drought levels ( $-0.53$  MPa). Predawn respiration ( $R_{PD}$ ) was extremely sensitive to drought stress, where  $R_{PD}$  was at a maximum ( $3.67 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) on July 31 and had declined 25% by August 4 (4 days without



**Fig. 2.** The effect of progressive drought stress and recovery on maximum quantum yield of photosystem II ( $F_v/F_m$ ; A) and net photosynthesis ( $A_N$ ; B) at Tifton, Georgia. Water was withheld beginning at first flower (July 31) and continuing until peak bloom (August 21). Thereafter, plants were returned to well-watered conditions for four days prior to a final recovery measurement (August 25). Data represent means and standard errors ( $n = 12$ ).

water).  $R_{PD}$  continued to decline throughout the drought period, reaching a minimum on August 19. In contrast with  $\Psi_{PD}$ ,  $R_{PD}$  did not recover to pre-stress levels when returned to well-watered conditions, where  $R_{PD}$  values were 55% lower on August 25 (following a four day recovery) than on July 31 (Fig. 1).

Maximum quantum yield of PSII ( $F_v/F_m$ ) demonstrated minimal variation throughout the drought period (Fig. 2A). Specifically,  $F_v/F_m$  was 0.83 on July 31 and had increased only 1.2% by August 21 (the last day of the stress period). The lowest value observed throughout the study ( $F_v/F_m = 0.82$  on August 7) was only 2.3% lower than the highest value observed ( $F_v/F_m = 0.84$  on August 21). Net photosynthesis ( $A_N$ ; 2B) did not decline significantly during the first week of drought (mean  $A_N = 31.3 \mu\text{mol m}^{-2} \text{s}^{-1}$  on August 4 and 7); however,  $A_N$  did decline significantly by the last day of the drought stress period. Specifically,  $A_N$  was 19.5% lower on August 21 ( $23.7 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) than on July 31 ( $29.6 \mu\text{mol m}^{-2} \text{s}^{-1}$ ), and  $A_N$  increased to  $28.1 \mu\text{mol m}^{-2} \text{s}^{-1}$  following the four-day recovery period.

To quantify the relationship between plant water status on  $R_{PD}$ ,  $F_v/F_m$ , and  $A_N$ , the average daily  $\Psi_{PD}$  ( $n = 12$  for each sample date) was plotted versus average daily values for each of the aforementioned parameters. No significant relationship was observed between  $\Psi_{PD}$  and  $F_v/F_m$  (data not shown); however,  $A_N$  and  $R_{PD}$  were strongly dependent upon  $\Psi_{PD}$  (Fig. 3). For example, the relationship between  $A_N$  and  $\Psi_{PD}$  was best described by a second-order polynomial function ( $P < 0.001$ ;  $r^2 = 0.86$ ), where  $A_N$  was optimal at  $\Psi_{PD} = -0.67$  MPa and declined with additional decreases in  $\Psi_{PD}$ .



**Table 1**  
Average predawn water potential ( $\Psi_{PD}$ ) and respiration ( $R_{PD}$ ) from first flower to peak bloom and average fiber yield at two locations, under multiple irrigation regimes (Only one irrigation method was used at the Tifton location.), and for three different cultivars. Data are means ( $n=4$ ) and values not sharing a common letter within a given column and location are significantly different ( $P<0.05$ ). No cultivar main effect or interaction between cultivar and irrigation were observed, but cultivar averages are shown below to provide additional information.

Location	Treatment	Cultivar	Leaf $\Psi_{PD}$ (MPa)	Leaf $R_{PD}$ ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ )	Fiber yield ( $\text{kg ha}^{-1}$ )	
Camilla	T1	FM 1944	-0.55 <sup>a</sup>	3.67 <sup>a</sup>	1908 <sup>a</sup>	
		PHY 499	-0.59 <sup>a</sup>	3.60 <sup>a</sup>	2058 <sup>a</sup>	
		PHY 499	-0.55 <sup>a</sup>	3.39 <sup>a</sup>	1897 <sup>a</sup>	
	T2	FM 1944	-0.57 <sup>a</sup>	3.35 <sup>a</sup>	2093 <sup>a</sup>	
		PHY 499	-0.72 <sup>b</sup>	2.91 <sup>b</sup>	1463 <sup>b</sup>	
		PHY 499	-0.71 <sup>b</sup>	3.09 <sup>b</sup>	1604 <sup>b</sup>	
	T4	FM 1944	-0.82 <sup>cb</sup>	2.89 <sup>b</sup>	1164 <sup>c</sup>	
		PHY 499	-0.82 <sup>cb</sup>	2.57 <sup>b</sup>	1118 <sup>c</sup>	
		PHY 499	-0.81 <sup>c</sup>	2.96 <sup>b</sup>	852 <sup>d</sup>	
	T5	FM 1944	-0.81 <sup>c</sup>	2.73 <sup>b</sup>	782 <sup>d</sup>	
		PHY 499	-0.81 <sup>c</sup>	2.73 <sup>b</sup>	782 <sup>d</sup>	
		PHY 499	-0.81 <sup>c</sup>	2.73 <sup>b</sup>	782 <sup>d</sup>	
	Tifton	-	FM 1944	-0.79 <sup>a</sup>	2.37 <sup>a</sup>	798 <sup>a</sup>
			PHY 499	-0.78 <sup>a</sup>	2.39 <sup>a</sup>	845 <sup>a</sup>
			DP 1050	-0.82 <sup>a</sup>	2.56 <sup>a</sup>	876 <sup>a</sup>

$R_{PD}$  was more sensitive to drought than  $A_N$  as a strong, linear relationship was observed between  $R_{PD}$  and  $\Psi_{PD}$  ( $P<0.001$ ;  $r^2=0.96$ ). Specifically, as  $R_{PD}$  was maximal at  $\Psi_{PD}=-0.54$  MPa and declined linearly with decreasing  $\Psi_{PD}$ .

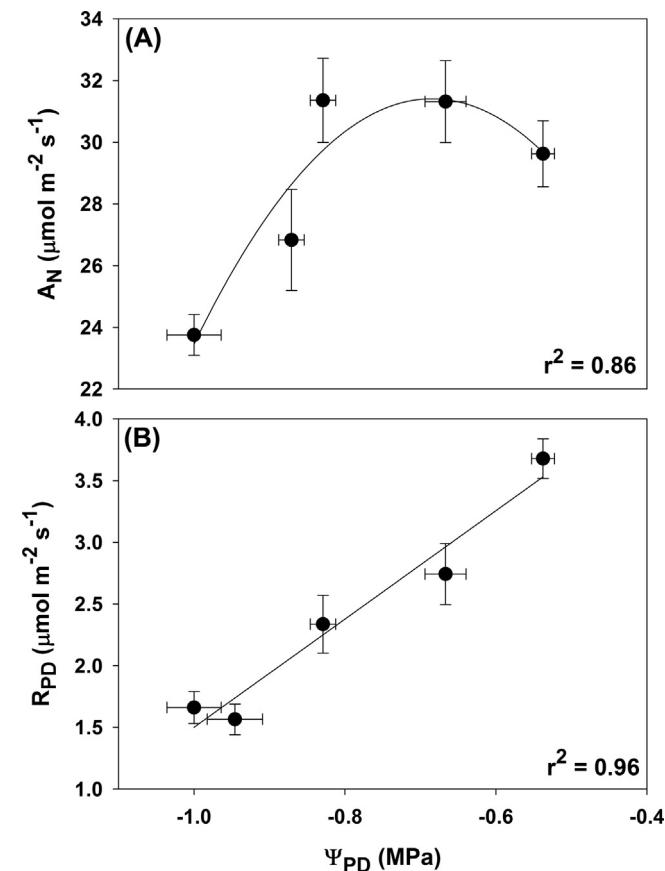
#### Characterization of study sites

Based on the sensitivity of  $R_{PD}$  to progressive drought from first flower to peak bloom at the Tifton location (Figs. 1 and 3), the relationship between  $R_{PD}$  and fiber yield under a range of plant water

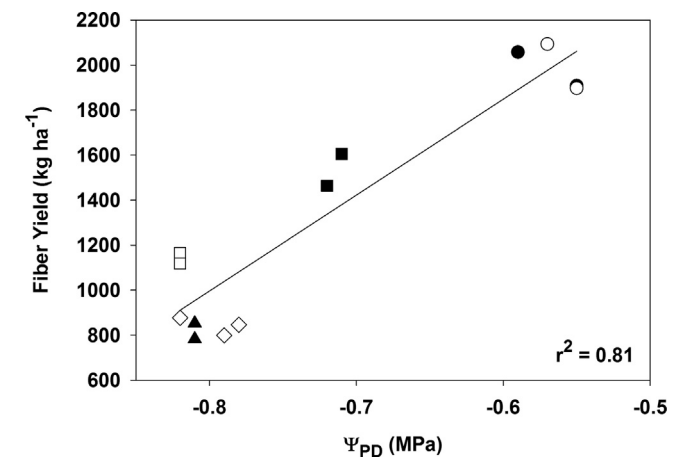
status was assessed by first determining the average  $R_{PD}$  and  $\Psi_{PD}$  from first flower to peak bloom and average fiber yield for each cultivar and irrigation treatment at both locations (Tifton and Camilla); these data are presented in Table 1. Neither the cultivar main effect (Tifton and Camilla) nor the interaction between cultivar and irrigation treatment (Camilla only) were significant. However, at the Camilla location, the only site where multiple irrigation treatments were imposed, irrigation treatment significantly affected all three parameters ( $P<0.001$ ). When averaged from first flower to peak bloom,  $\Psi_{PD}$  was significantly higher for T1 and T2 than all other treatments;  $\Psi_{PD}$  for T4 and T5 was significantly lower than for all other treatments.  $R_{PD}$  formed two statistically distinct groups, where T1 and T2 had significantly higher  $R_{PD}$  rates than the remaining treatments. Fiber yield demonstrated the following response to irrigation treatment: T1 = T2 > T3 > T4 > T5.

#### Effects of $\Psi_{PD}$ and $R_{PD}$ on yield

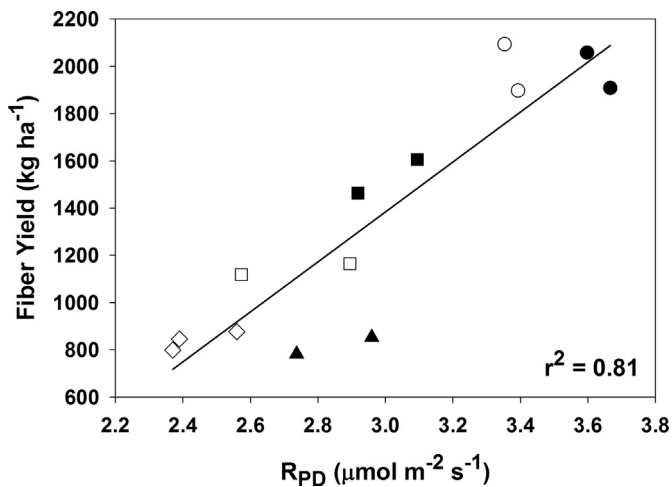
To assess the impact of plant water status from first flower to peak bloom on fiber yield in the current study, data were first averaged from all replicate plots for a given location  $\times$  irrigation treatment  $\times$  cultivar (Table 1). Subsequently, fiber yield was plotted versus  $\Psi_{PD}$  and regression analysis demonstrated a strong, positive



**Fig. 3.** The relationship between average predawn water potential ( $\Psi_{PD}$ ), net photosynthesis ( $A_N$ ; A) and predawn respiration ( $R_{PD}$ ; B) during the three week drought period presented in Figs. 1 and 2 (from July 31 to August 21) from the Tifton, Georgia location. Data points represent means and standard errors ( $n=12$ ) for each sample date during the drought stress period.



**Fig. 4.** Average predawn water potential ( $\Psi_{PD}$ ) during the first two weeks of flowering versus final fiber yield of cotton. Each data point represents the mean of 4 replicate plots (the average value for a given cultivar and irrigation treatment), and  $\Psi_{PD}$  was measured a minimum of six times (in two or three day intervals) from first flower to peak bloom (between two and three weeks later). Data were combined from the Tifton and Camilla, GA field sites. Regression analysis was conducted using a linear function. Symbols for each treatment-location are as follows: ● = T1-Camilla; ○ = T2-Camilla; ■ = T3-Camilla; □ = T4-Camilla; ▲ = T5-Camilla; ◇ = Tifton.



**Fig. 5.** Average predawn respiration rates ( $R_{PD}$ ) during the first two weeks of flowering versus final fiber yield of *Gossypium hirsutum*. Each data point represents the mean of 4 replicate plots (the average value for a given cultivar and irrigation treatment), and  $R_{PD}$  was measured a minimum of two times during the flowering period, once at first flower and once at peak bloom (two to three weeks later). Data were combined from the Tifton and Camilla, GA field sites. Regression analysis was conducted using linear regression. Symbols for each treatment-location are as follows: ● = T1-Camilla; ○ = T2-Camilla; ■ = T3-Camilla; □ = T4-Camilla; ▲ = T5-Camilla; ◇ = Tifton.

linear relationship between the aforementioned variables (Fig. 4;  $P < 0.001$ ;  $r^2 = 0.81$ ). Thus the majority of yield variability observed in the current study was water-induced. A similar approach was utilized to quantify the relationship between predawn respiration rates and lint yield. In this instance, the dependence of yield on respiration rate was best described by a linear function, and a strong, positive relationship between respiration rates and yield was observed (Fig. 5;  $P < 0.001$ ;  $r^2 = 0.81$ ). Thus, higher predawn respiration rates were indicative of higher yields when yield variability was predominantly water-induced.

## Discussion

The results presented in Figs. 1–3 supported the hypothesis that  $R_{PD}$  would be more sensitive to the onset of drought than photosynthetic processes. For example, within four days after drought was imposed,  $R_{PD}$  had declined 25% as  $\Psi_{PD}$  declined only 0.13 MPa relative to pre-drought levels (Fig. 1). Furthermore,  $R_{PD}$  continued to decline thereafter with progressive declines in  $\Psi_{PD}$ , where a strong, linear relationship between  $R_{PD}$  and  $\Psi_{PD}$  was observed when data were analyzed during the drought stress period (Fig. 3). To our knowledge, there are no prior reports on the sensitivity of  $R_{PD}$  to progressive drought for field-grown *G. hirsutum*. Pallas et al. (1967) demonstrated an initial decline in  $R$  (measured during afternoon hours) in response to progressive drought followed by an increase in  $R$  near the end of the drought period (11 d) for greenhouse-grown cotton. Other authors have suggested that this biphasic response of  $R$  to drought, an initial decline at the onset of drought followed by an increase under severe water deficit, is fairly common (Atkin and Macherel, 2009; Flexas et al., 2006). Thus, differences in drought stress timing, duration, and severity likely explain some of the apparently conflicting results in the literature (Atkin and Macherel, 2009; Chastain et al., 2014; Massacci et al., 2008; Pallas et al., 1967; Zhang et al., 2011). An inherent limitation to the experimental design at the Tifton site is the lack of a well-watered control. Therefore, it could be argued that the decline in  $R_{PD}$  observed at the Tifton location during the progressive drought period could be the result of normal phenological

changes in  $R_{PD}$  rather than a response to drought stress. It should be noted, however, that average  $R_{PD}$  measured at peak bloom (August 8;  $3.9 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) for well-watered plants at the Camilla location (T1 and T2 were considered well-watered based upon the yield and water potential data presented in Table 1) was actually higher than  $R_{PD}$  measured at first flower (July 26;  $2.6 \mu\text{mol m}^{-2} \text{s}^{-1}$ ), providing no evidence for a phenology-dependent decline in  $R_{PD}$  from first flower to peak bloom. In contrast with  $R_{PD}$ , maximum quantum yield of photosystem II ( $F_v/F_m$ ) was relatively insensitive to drought, where  $F_v/F_m \geq 0.823$  for all sample dates assessed, and  $F_v/F_m$  was greatest following three weeks of drought (Fig. 2). Although primary photochemistry can be negatively impacted under drought in some species (soybean; Zhang et al., 2011), most available reports with field-grown cotton indicate that photosynthetic efficiency of PSII, in light or dark-adapted leaves, is either unaffected or responds positively to moderate drought stress (Chastain et al., 2014; Kitao and Lei, 2007; Snider et al., 2013, 2014; Zhang et al., 2011), which agrees closely with the results presented in Fig. 2. Though not as sensitive to drought as  $R_{PD}$ ,  $A_N$  was substantially more sensitive to drought than PSII (Fig. 2). For example,  $A_N$  exhibited a numeric (though not statistically significant) increase at four and seven days after drought exposure compared with pre-drought conditions. Thereafter,  $A_N$  did eventually decline 20% by the last day of stress relative to the initial measurement date (Fig. 2). In contrast with  $R_{PD}$ ,  $A_N$  did not decline linearly with declines in  $\Psi_{PD}$  below well-watered conditions; instead, a quadratic relationship between the aforementioned parameters was observed, where  $A_N$  was not predicted to decline appreciably (5% for example) until  $\Psi_{PD} < -0.8$  MPa; this threshold is in close agreement with previous observations in our laboratory for field-grown cotton (Snider et al., 2014). Although  $R$  is often considered less sensitive to water deficit than  $A_N$  (Atkin and Macherel, 2009), the results of the current study (Figs. 1–3) do not support this view for field-grown *G. hirsutum* exposed to progressive drought stress. Based on the previous discussion, the relative sensitivity of the aforementioned processes to drought progression in cotton can be summarized as follows:  $R_{PD} > A_N > F_v/F_m$  or  $\Phi_{PSII}$ . Furthermore,  $R_{PD}$  and  $A_N$  responded differently to rewatering (Figs. 1 and 2). Specifically,  $A_N$  had increased to a level that was not significantly different than pre-drought  $A_N$  upon return to well-watered conditions for four days, but  $R_{PD}$  had not changed, suggesting a lasting drought-induced inhibition of  $R_{PD}$ .

Results presented in Fig. 4 to Fig. 5 supported the hypothesis that fiber yield would be dependent upon  $R_{PD}$  when yield variability was water induced. In previous studies in which  $R$  has been linked to yield in important agronomic crops, an inverse relationship between the two parameters is observed (Earl and Tollenaar, 1998; Peng et al., 2004). However, previous studies have not addressed the potential for  $R_{PD}$  to predict water-induced yield variability. By design, yield was strongly dependent upon plant water status during flowering (Fig. 4). In general, high respiration rates during flowering were associated with high yields, where a strong, positive, non-linear relationship was observed between fiber yield and  $R_{PD}$  (Fig. 5). Furthermore, the highest yields ( $2058 \text{ kg ha}^{-1}$ ) were observed at  $R_{PD} = 3.6 \mu\text{mol m}^{-2} \text{s}^{-1}$  and  $\Psi_{PD} = -0.59$  MPa when averaged from first flower to peak bloom. The lowest observed yield in the present study was observed at  $R_{PD} = 2.73 \mu\text{mol m}^{-2} \text{s}^{-1}$  and  $\Psi_{PD} = -0.81$  MPa. Readers should note that an increase in respiration rates was not observed with progressively lower  $\Psi_{PD}$  in the present study (Figs. 1 and 3; Table 1), as indicated by many other authors (reviewed in Atkin and Macherel, 2009). We do not argue that an increase in  $R_{PD}$  is likely under more severe drought stress than was observed in the current study. However, as noted above, the lowest yielding treatment in the current study produced an average yield that was  $1276 \text{ kg ha}^{-1}$  lower than the most well-watered treatment (Table 1). Thus, within the range of

water status that has a substantial impact on productivity in *G. hirsutum*, higher respiration rates should be predictive of higher yields.

The findings of the current study indicate for field-grown *G. hirsutum* that (1)  $R_{PD}$  is exceptionally sensitive to progressive drought and (2) average  $R_{PD}$  from first flower to peak bloom is highly predictive of water-induced yield variability. The possibility that genotypic variation in yield stability under drought can be explained by  $R_{PD} \times \Psi_{PD}$  interactions during flowering should be assessed in the future with diverse germplasm.

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